A novel thermosensitive chitosan for the treatment of degenerative intervertebral disc

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Statement of Purpose: Degeneration of the intervertebral disc (IVD) is the most common cause of back pain for aged people. IVD consists of two distinct anatomical zones: an outer annulus fibrousus (AF) and a central nucleus pulposus (NP). It is known that, with increasing age, the NP is transformed from a gelatinous tissue rich in proteoglycans into a more fibrous tissue with diminished proteoglycan content.[1] These convert of NP structure cause the outer AF to lose its normal lamellar arrangement and compromises the mechanical strength of the disc. Recent therapies for the treatment of IVD disease have been focused on the several surgical options, such as metallic implants, polymer scaffolds, and gene therapy, according to the patient's status. Among these therapies, injectable applications using hydrogel have received a considerable attention for IVD disease due to patient's comfort by ease of application.

In this study, our aims are to evaluate the potential for IVD treatment using injectable hydrogels with thermosensitivity based on the minimal invasive technique and investigate *in vitro* release profile of OP-1 (Osteogenic Protein-1), which is an effective growth factor for NP and AF regeneration.

Methods: As a first step for the preparation of chitosan based hydrogels, Pluronic® F127 was carboxylated with succinic anhydride to produce monocarboxy Pluronic® F127[2]. Then, monocarboxy Pluronic® was coupled with chitosan by EDC/NHS at room temperature for 24h.[2] The product was dialyzed against distilled water using a membrane (MWCO, 50kDa) for 3 days and finally lyophilized to give chitosan-Pluronic®. chitosan-g-NIPAAm as another injectable material was prepared by grafting NIPAAm onto chitosan backbone as we previously reported.[3]

The physico-chemical properties of these thermosensitive chitosan copolymers were characterized by FT-IR, ^{1}H-NMR, TGA and solubility test. Sol-gel transition behavior was investigated by the vial tilting method. The MTT assay with intervertebral disc NP cells was performed, including alginate gel as a control. For *in vitro* release study, OP-1 was simply mixed with an aqueous solution of chitosan copolymer at ambient temperature and the release profile of OP-1 from the hydrogel matrix was investigated at predetermined time interval.

Results / Discussion: The chemical structures of chitosan-Pluronic® and chitosan-g-NIPAAm were characterized by physico-chemical method. They demonstrated the unique characteristics of thermosensitive chitosan modified with Pluronic® or NIPAAm. Their aqueous solution showed the sol-gel transition behaviors around body temperature. The mechanical properties between these two hydrogels were similar but the water content was different after gelation as a result of water exclusion from chitosan-g-NIPAAm hydrogel. In the result of MTT assay after NP cell seeding, there are no significant differences between chitosan copolymers but chitosan-Pluronic® was more biocompatible than other groups, respectively.

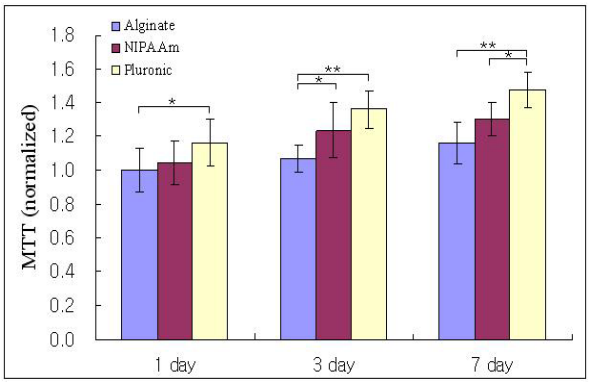

Figure 1. The cytotoxicity of thermosensitive chitosan

OP-1 has been shown to accelerate the formation of new bone and NP in numerous preclinical and clinical studies.[4] OP-1 has shown the sustained release from chitosan-Pluronic® gel. The *in vivo* animal study is ongoing.

Conclusions: In summary, two chitosan based thermosensitive polymers as a cell-supporting matrix and drug carrier were investigated. Obtained results suggest that these injectable hydrogels can be very useful as a new method for IVD treatment, especially NP and AF regeneration.

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